

FILE 'BIOSIS, MEDLINE, EMBASE, EMBAL, SCISEARCH, BIOTECHDS, CAPLUS'
ENTERED AT 15:22:37 ON 06 OCT 2003

L1	5033 S BISULFITE? AND METHYL?
L2	23 S L1 AND (CY3? AND CY5)
L3	17 DUP REM L2 (6 DUPLICATES REMOVED)
L4	16 S L3 AND (PCR? OR AMPLIF?)
L5	1 S L1 AND (FLOURESC?)
L6	153 S L1 AND (PURIFICATION? OR SEPARATION?)
L7	151 S L6 NOT L3
L8	21 S L7 AND (OLIGONUCLEOTIDE? OR SOLID?)
L9	20 DUP REM L8 (1 DUPLICATE REMOVED)
L10	20 S L9 NOT L2

L Number	Hits	Search Text	DB	Time stamp
1	187	bisulfite and (methy\$4 SAME cytosine)	USPAT; US-PGPUB; EPO; JPO; DERWENT; IBM_TDB	2003/10/06 14:31
2	165	((bisulfite and (methy\$4 SAME cytosine)) and (PCR or amplif\$8))	USPAT; US-PGPUB; EPO; JPO; DERWENT; IBM_TDB	2003/10/06 14:31
3	103	((bisulfite and (methy\$4 SAME cytosine)) and (PCR or amplif\$8)) and (fluorescent\$3)	USPAT; US-PGPUB; EPO; JPO; DERWENT; IBM_TDB	2003/10/06 14:31
4	6	((bisulfite and (methy\$4 SAME cytosine)) and (PCR or amplif\$8)) and (fluorescent\$3)	USPAT; US-PGPUB; EPO; JPO; DERWENT; IBM_TDB	2003/10/06 14:31
5	109	((bisulfite and (methy\$4 SAME cytosine)) and (PCR or amplif\$8)) and fluorescent\$3	USPAT; US-PGPUB; EPO; JPO; DERWENT; IBM_TDB	2003/10/06 14:32
6	20	((bisulfite and (methy\$4 SAME cytosine)) and (PCR or amplif\$8)) and fluorescent\$3 and (cy3 and cy5)	USPAT; US-PGPUB; EPO; JPO; DERWENT; IBM_TDB	2003/10/06 14:56
7	2	wo near "9928498"	USPAT; US-PGPUB; EPO; JPO; DERWENT; IBM_TDB	2003/10/06 14:57

FILE 'BIOSIS, MEDLINE, EMBASE, EMBAL, SCISEARCH, BIOTECHDS, CAPLUS'
ENTERED AT 11:53:13 ON 06 OCT 2003

L1 7168 S **METHYLATION** (S) **CYTOSINE**?
L2 17 S L1 AND (**BISULFATE**?)
L3 16 DUP REM L2 (1 DUPLICATE REMOVED)
L4 8 S L3 AND (**PCR**? OR **AMPLF**?)
L5 0 S L4 AND **CY3**?
L6 8 S L3 NOT L4
L7 72 S SEPARATION AND (**METHYLAT**? (S) **CYTOSINE**?)
L8 28 S L7 AND (**ELECTROPHORESIS** OR **HPLC**? OR **CPE**? OR
(CAPLLIARY ADJ1
L9 0 S L8 AND (**CY3** OR **CY5**)
L10 0 S L9 AND (**DCTP**? OR **DGTP**?)
L11 0 S L8 AND (**DCTP**? OR **DGTP**?)
L12 18 DUP REM L8 (10 DUPLICATES REMOVED)
L13 916 S **CY3** AND **CY5**
L14 21 S L13 AND (**METHYL**? AND **CYTOSINE**?)
L15 8 S L4 AND **BISULFATE**?
L16 8 DUP REM L15 (0 DUPLICATES REMOVED)
L17 0 S L14 AND **BISULFATE**?
L18 19 DUP REM L14 (2 DUPLICATES REMOVED)

L4 ANSWER 2 OF 8 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.
on STN

ACCESSION NUMBER: 1997:87207 BIOSIS

DOCUMENT NUMBER: PREV199799378920

TITLE: Rapid analysis of DNA **methylation** using new restriction
enzyme sites created by bisulfite modification.

AUTHOR(S): Sadri, Ramin; Hornsby, Peter J. (1)

CORPORATE SOURCE: (1) Dep. Cell Biol., Baylor Coll. Med., 1 Baylor Plaza
M320, Houston, TX 77030 USA

SOURCE: Nucleic Acids Research, (1996) Vol. 24, No. 24, pp.
5058-5059.

ISSN: 0305-1048.

DOCUMENT TYPE: Article

LANGUAGE: English

AB Bisulfite converts non-**methylated cytosine** in DNA to uracil
leaving 5-methyl**cytosine** unaltered. Here, predicted changes in restriction
enzyme sites following reaction of genomic DNA with bisulfite and
amplification of the product by the polymerase chain reaction (**PCR**
) were used to assess the **methylation** of CpG sites. This
procedure differs from conventional DNA **methylation** analysis by
methylation-sensitive restriction enzymes because it does not rely
on an absence of cleavage to detect **methylated** sites, the two strands of.
. . different restriction enzyme sites and may be differentially

analyzed, and closely related sequences may be separately analyzed by using specific **PCR** primers.

L4 ANSWER 6 OF 8 BIOTECHDS COPYRIGHT 2003 THOMSON DERWENT/ISI on STN

ACCESSION NUMBER: 1997-02964 BIOTECHDS

TITLE: **Methylation** analysis using **bisulfate** genomic sequencing: application to small numbers of intact cells; mouse Xist gene **methylation** profile by polymerase chain reaction

AUTHOR: McDonald L E; *Kay G F

CORPORATE SOURCE: Queensland-Inst.Med.Res.; Roy.Brisbane-Hosp.

LOCATION: Queensland Institute of Medical Research, Post Office Royal Brisbane Hospital, Queensland, 4029, Australia.
Email: grahamk@qimr.edu.au

SOURCE: BioTechniques; (1997) 22, 2, 272,273

CODEN: BTNQDO

ISSN: 0736-6205

DOCUMENT TYPE: Journal

LANGUAGE: English

TI **Methylation** analysis using **bisulfate** genomic sequencing: application to small numbers of intact cells; mouse Xist gene **methylation** profile by polymerase chain reaction

AB **Bisulfate** genomic sequencing was applied to samples containing 50-200 intact cells to determine the **methylation** status of each **cytosine** residue in a defined sequence for individual strands of genomic DNA. Bisulfite-modified DNA is subjected to polymerase chain reaction (**PCR**) to yield products in which uracil residues are **amplified** as thymine and 5-methyl**cytosine** residues only are **amplified** as **cytosine**. This method allows the investigation of **methylation** in the control of gene expression in germ cells, embryogenesis, inherited diseases and cancers, when samples are limiting. This method was used to generate **methylation** profiles for a mouse Xist gene (569 bp DNA fragment) in mouse germ cells and during implantation development in order to study the role of **methylation** in the genomic imprinting of this gene. Genomic DNA was prepared from cells treated with a lysis buffer containing protease-K and guanidine hydrochloride. Isolated genomic DNA was modified with **bisulfate**, denatured by restriction endonuclease digestion and subjected to touchdown **PCR**. This method resulted in complete conversion of **cytosine** residues for all samples analyzed. (12 ref)

L6 ANSWER 8 OF 8 CAPLUS COPYRIGHT 2003 ACS on STN

ACCESSION NUMBER: 1999:740564 CAPLUS

DOCUMENT NUMBER: 132:246749

TITLE: Analysis of in vivo **methylation**
AUTHOR(S): Dahl, Hans-Henrik M.; Hutchison, Wendy M.
CORPORATE SOURCE: The Murdoch Institute, Royal Children's Hospital,
Melbourne, Australia
SOURCE: Methods in Molecular Biology (Totowa, New Jersey)
(2000), 130(Transcription Factor Protocols), 47-57
CODEN: MMBIED; ISSN: 1064-3745
PUBLISHER: Humana Press Inc.
DOCUMENT TYPE: Journal; General Review
LANGUAGE: English
REFERENCE COUNT: 19 THERE ARE 19 CITED REFERENCES
AVAILABLE FOR THIS

RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

AB A review with 19 refs. A no. of methods are available to detect **methylated cytosines** (as 5-methylcytosine, 5-MeC) in DNA and most are based on the use of **methylation**-sensitive restriction enzymes or genomic sequencing protocols. Recently, a simple and efficient genomic sequencing technol. has been developed. It is based in the observation that sodium **bisulfate**, followed by alk. treatment, converts **cytosine** residues in single stranded DNA to uracil under condition where 5-MeC is unreactive. Modifications of the bisulfite genomic sequencing technique are outlined.

IT 7681-38-1, Sodium **bisulfate**

RL: ARG (Analytical reagent use); ANST (Analytical study); USES (Uses)
(anal. of in vivo **methylation** by bisulfite genomic sequencing)

L6 ANSWER 6 OF 8 BIOTECHDS COPYRIGHT 2003 THOMSON DERWENT/ISI
on STN

ACCESSION NUMBER: 1999-03515 BIOTECHDS

TITLE: Detecting differences in **methylation** of DNA at specific CpG
sites;
by polymerase chain reaction, used for cancer diagnosis

AUTHOR: Gonzalgo M L; Jones P A; Liang G

PATENT ASSIGNEE: Univ.Southern-California

LOCATION: Los Angeles, CA, USA.

PATENT INFO: WO 9856952 17 Dec 1998

APPLICATION INFO: WO 1998-US11896 9 Jun 1998

PRIORITY INFO: US 1997-49231 9 Jun 1997

DOCUMENT TYPE: Patent

LANGUAGE: English

OTHER SOURCE: WPI: 1999-080918 [07]

AB A method for determining DNA **methylation** patterns at **cytosine** sites is claimed. It involves reacting DNA from a sample to be assayed, with sodium **bisulfate**, to convert **unmethylated cytosine** to uracil, leaving 5-methylcytosine unconverted. DNA primers specific for the converted sample are then used

to perform a polymerase chain. . . be measured by incorporating various 32P labeled dNTPs. Also claimed is a Ms-SNuPE primer sequence that hybridizes with a specific **cytosine** codon in a target CpG site, and that corresponds to a frequently **hypermethylated** gene sequence just upstream of the target. . . site by locating a **hypermethylated** CpG island in a somatic gene derived from malignant tissue. This is used to detect **methylation** changes associated with oncogenic transformation, and therefore to detect cancer. (19pp)

L12 ANSWER 8 OF 18 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC. on STN

DUPLICATE 5

ACCESSION NUMBER: 2000:478524 BIOSIS

DOCUMENT NUMBER: PREV200000478524

TITLE: Rapid quantification of DNA **methylation** by high performance **capillary electrophoresis**.

AUTHOR(S): Fraga, Mario F.; Rodriguez, Roberto; Canal, Maria Jesus (1)

CORPORATE SOURCE: (1) Dpto. B.O.S. (Lab. Fisiologia Vegetal), Universidad de Oviedo, C/Catedratico Rodrigo Uria s/n, E-33071, Oviedo Spain

SOURCE: **Electrophoresis**, (August, 2000) Vol. 21, No. 14, pp. 2990-2994. print.
ISSN: 0173-0835.

DOCUMENT TYPE: Article

LANGUAGE: English

SUMMARY LANGUAGE: English

TI Rapid quantification of DNA **methylation** by high performance **capillary electrophoresis**.

AB The actual methods to evaluate total DNA **methylation** based on high performance liquid chromatography (**HPLC**) are long and tedious due to the specific running buffers required. In this work, a new open-tube **capillary electrophoresis** system has been applied to the separation of acid hydrolyzed genomic DNA and so, to the evaluation of genomic DNA **methylation**. Several running conditions were tested but separation of **cytosine** and 5-methyl-**cytosine** was only possible by sodium dodecyl sulfate (SDS) micelle system. The importance of sample dissolution preparation has also been demonstrated. The results of this study open up the possibility of quantification of the relative **methylation** degree of rapid genomic DNA by a simple method based on high performance **capillary electrophoresis** (HPCE).

L12 ANSWER 9 OF 18 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC. on STN

ACCESSION NUMBER: 2000:197982 BIOSIS

DOCUMENT NUMBER: PREV200000197982

TITLE: C5 **cytosine methylation** at CpG sites
enhances sequence selectivity of mitomycin C-DNA bonding.

AUTHOR(S): Li, Ven-Shun; Reed, Matthew; Zheng, Yi; Kohn, Harold; Tang,
Moon-shong (1)

CORPORATE SOURCE: (1) Department of Environmental Medicine, New York
University, Tuxedo, NY, 10987 USA

SOURCE: Biochemistry, (March 14, 2000) Vol. 39, No. 10, pp.
2612-2618.

ISSN: 0006-2960.

DOCUMENT TYPE: Article

LANGUAGE: English

SUMMARY LANGUAGE: English

TI C5 **cytosine methylation** at CpG sites enhances sequence
selectivity of mitomycin C-DNA bonding.

AB. . . the degree of UvrABC cutting represents the extent of drug-DNA
bonding. Using this method we determined the effect of C5 **cytosine
methylation** on the DNA monoalkylation by MC and the related
analogues N-methyl-7-methoxyaziridinomitosene (MS-NMA) and
10-decarbamoyletomycin C (DC-MC). We have found that C5 **cytosine
methylation** at CpG sites greatly enhances MC and MS-NMA DNA adduct
formation at those sites while reducing adduct formation at non-CpG
sequences. In contrast, although DC-MC DNA bonding at CpG sites is greatly
enhanced by CpG **methylation**, its bonding at non-CpG sequences is
not appreciably affected. These cumulative results suggest that C5
cytosine methylation at CpG sites enhances sequence
selectivity of drug-DNA bonding. We propose that the **methylation**
pattern and status (hypo- or hyper**methylation**) of genomic DNA may
determine the cells' susceptibility to MC and its analogues, and. . .

L12 ANSWER 10 OF 18 SCISEARCH COPYRIGHT 2003 THOMSON ISI on STN
ACCESSION NUMBER: 2001:109997 SCISEARCH

THE GENUINE ARTICLE: 393BM

TITLE: AFLP-based detection of DNA **methylation**

AUTHOR: Xu M L (Reprint); Li X Q; Korban S S

CORPORATE SOURCE: Univ Illinois, Dept Nat Resources & Environm Sci, Urbana,
IL 61801 USA; Univ Illinois, Dept Nat Resources & Environm
Sci, Urbana, IL 61801 USA

COUNTRY OF AUTHOR: USA

SOURCE: PLANT MOLECULAR BIOLOGY REPORTER, (DEC 2000) Vol.
18, No.

4, pp. 361-368.

Publisher: INT SOC PLANT MOLECULAR BIOLOGY, UNIV

GEORGIA,

DEPT BIOCHEMISTRY, ATHENS, GA 30602 USA.

ISSN: 0735-9640.

DOCUMENT TYPE: Article; Journal

LANGUAGE: English

REFERENCE COUNT: 17

ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB By using the isoschizomers Hpa II and Msp I which display differential sensitivity to **cytosine methylation**, a modified **amplified** fragment length polymorphism (AFLP) technique has been developed to investigate DNA **methylation** profiles in eukaryotic organisms. Genomic DNA was digested with a mixture of EcoR I and one of the isoschizomers, and ligated to oligonucleotide adapters. After two rounds of selective **PCR amplification**, followed by DNA separation on a Long Ranger gel **electrophoresis**, a subset of restriction fragments can be displayed on an X-ray film. Comparison of AFLP banding patterns between Hpa II and Msp I revealed the extent of DNA **methylation**. The technique has been successfully applied in this study to investigate DNA **methylation** profiles of apple (*Malus domestica* cv. Gala) genomic DNA extracted from leaves of field-grown adult trees and in vitro-grown shoot cultures. The results showed that up to 25 percent of AFLP bands were derived from **methyalted** sequences, and among those, a few bands unique to either adult trees or in vitro shoots were observed. These results demonstrated that this protocol is effective in identifying **methyalted** DNA profiles.

STP KeyWords Plus (R): **CYTOSINE METHYLATION**; RESISTANCE; GENE; **AMPLIFICATION**; SEQUENCES; PATTERNS; MARKERS; FUNGI

L12 ANSWER 11 OF 18 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC. on STN

ACCESSION NUMBER: 2000:339856 BIOSIS

DOCUMENT NUMBER: PREV200000339856

TITLE: **PCR amplification** in bisulfite methyl**cytosine** mapping in the GC-rich promoter region of amyloid precursor protein gene in autopsy human brain.

AUTHOR(S): Nagane, Yuriko; Utsugisawa, Kimiaki; Tohgi, Hideo (1)

CORPORATE SOURCE: (1) Department of Neurology, Iwate Medical University, 19-1 Uchimaru, Morioka, Iwate, 020-8505 Japan

SOURCE: Brain Research Protocols, (April, 2000) Vol. 5, No. 2, pp. 167-171. print.

ISSN: 1385-299X.

DOCUMENT TYPE: Article

LANGUAGE: English

SUMMARY LANGUAGE: English

AB. . . in DNA 5-methyldeoxycytidine pattern influence gene expression for certain mammalian genes in development, differentiation, carcinogenesis, and aging. Detection of DNA **methylation** at the promoter region, which generally represses transcription activity, is one important element in studying changes in molecular expression with aging and age-associated

disorders. Bisulfite genomic sequencing is a useful method for mapping **methylated cytosines**. However, **PCR amplification** for bisulfite-treated DNA does not yield a sufficient amount of products that have a sufficient level of. . .

IT Methods & Equipment

ABI 373 STRETCH autosequencer: Perkin Elmer cetus, laboratory equipment; Horizon 11.14 gel **electrophoresis** apparatus: Gibco BRL, laboratory equipment; **PCR** [polymerase chain reaction]: DNA **amplification**, DNA **amplification** method, in-situ recombinant gene expression detection, sequencing. . . equipment; bisulfite genomic sequencing: Recombinant DNA Technology, gene sequencing method, sequencing techniques; bisulfite methyl**cytosine** mapping: gene mapping, gene mapping method; **electrophoresis**: electrophoretic techniques, separation method; ethidium bromide staining: staining method, staining/visualization

L12 ANSWER 13 OF 18 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC. on STN

ACCESSION NUMBER: 2000:200213 BIOSIS

DOCUMENT NUMBER: PREV200000200213

TITLE: Opposite effects of **methylated** and fluorinated **cytosine** on the reactivity of CpG with benzo(A)pyrene diolepoxide (BPDE) in oligonucleotides.

AUTHOR(S): Tomasz, Maria (1); Das, Arunangshu (1)

CORPORATE SOURCE: (1) Department of Chemistry, Hunter College, City University of New York, 695 Park Ave, New York, NY, 10021 USA

SOURCE: Abstracts of Papers American Chemical Society, (2000) Vol. 219, No. 1-2, pp. TOXI 73.
Meeting Info.: 219th Meeting of the American Chemical Society. San Francisco, California, USA March 26-30, 2000
American Chemical Society
. ISSN: 0065-7727.

DOCUMENT TYPE: Conference

LANGUAGE: English

SUMMARY LANGUAGE: English

TI Opposite effects of **methylated** and fluorinated **cytosine** on the reactivity of CpG with benzo(A)pyrene diolepoxide (BPDE) in oligonucleotides.

IT . . .

(Biochemistry and Molecular Biophysics); Toxicology

IT Diseases

cancer: neoplastic disease

IT Chemicals & Biochemicals

CpG; DNA; benzo[a]pyrene diolepoxide; fluorinated **cytosine**; **methylated cytosine**; oligonucleotides; p53

IT Alternate Indexing
Neoplasms (MeSH)
IT Methods & Equipment
HPLC [high performance liquid chromatography]:
separation method
IT Miscellaneous Descriptors
gene mutation; Meeting Abstract

L12 ANSWER 18 OF 18 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS
INC. on STN

DUPLICATE 6

ACCESSION NUMBER: 1985:406807 BIOSIS

DOCUMENT NUMBER: BA80:76799

TITLE: ANALYSIS OF PRODUCTS OF DNA MODIFICATION BY
METHYLASES A
PROCEDURE FOR THE DETERMINATION OF 5 METHYLCYTOSINE
AND N-4
METHYLCYTOSINE IN DNA.

AUTHOR(S): BUTKUS V V; KLIMASAUŠKAS S J; JANULAITIS A A
CORPORATE SOURCE: INSTITUTE OF APPLIED ENZYMOLOGY, 232028
VILNIUS, FERMENTU

8, LITHUANIAN SSR, USSR.

SOURCE: ANAL BIOCHEM, (1985) 148 (1), 194-198.

CODEN: ANBCA2. ISSN: 0003-2697.

FILE SEGMENT: BA; OLD

LANGUAGE: English

AB Although many different methods are used for the identification of
methylated heterocyclic bases in DNA not all of them possess the
ability to discriminate N4-methyl**cytosine** (m4C) and 5-methyl**cytosine**
(m5C). Therefore, some of the methods need reexamination. This paper
reinvestigates some chromatographic systems TLC, paper chromatography and
electrophoresis) most widely used in the analysis of minor bases
occurring in nucleic acids according to their ability to separate m4C. .
. sample and a chromatographic system for its analysis was developed. The
recommended chromatographic systems may be used for the simultaneous
separation of not only m4C and m5C, but also both
methylated cytosines together with N6-methyladenine and
7-methylguanine.

L18 ANSWER 5 OF 19 BIOTECHDS COPYRIGHT 2003 THOMSON
DERWENT/ISI on STN

ACCESSION NUMBER: 2003-02844 BIOTECHDS

TITLE: Analyzing **methylation** of **cytosine** bases
in genomic DNA, by chemically treating DNA to convert
cytosine to uracil, **amplifying** DNA and hybridizing
with fluorophore labeled probes, and detecting fluorescence

polarization;

DNA primer, DNA probe, DNA chip and bioinformatics for
high throughput screening and disease diagnosis

AUTHOR: BERLIN K

PATENT ASSIGNEE: EPIGENOMICS AG

PATENT INFO: WO 2002061123 8 Aug 2002

APPLICATION INFO: WO 2002-EP922 29 Jan 2002

PRIORITY INFO: DE 2001-1004937 29 Jan 2001; DE 2001-1004937 29 Jan 2001

DOCUMENT TYPE: Patent

LANGUAGE: English

OTHER SOURCE: WPI: 2002-674824 [72]

TI Analyzing **methylation** of **cytosine** bases in genomic

DNA, by chemically treating DNA to convert **cytosine** to uracil,
amplifying DNA and hybridizing with fluorophore labeled probes, and
detecting fluorescence polarization;

DNA primer, DNA probe, DNA chip. . .

AB DERWENT ABSTRACT:

NOVELTY - Analyzing **methylation** of specific **cytosine**
bases in genomic DNA samples, comprising chemically treating DNA to
convert **cytosine** to uracil, **amplifying** treated DNA using
oligonucleotide primer, contacting **amplified** sequence (AS) with
fluorophore labeled oligonucleotide probes, hybridizing fluorophore
labeled probes to AS, and detecting fluorescence polarization of labeled
probes, is new.

DETAILED DESCRIPTION - Analysis (M) of **methylation** of
specific **cytosine** bases in genomic DNA samples, comprising: (a)
chemically treating the genomic DNA for converting **cytosine** to
uracil or a similar acting base regarding the base pairing behavior in
the DNA duplex, 5 methyl**cytosine** remains unmodified; (b) the
chemically treated DNA is **amplified** using at least one oligonucleotide
(type A) as primer in a. . . one or more pairs of oligonucleotides
(type B), which hybridize to the positions which are to be examined
regarding their **methylation** status in the genomic DNA sample in
which one oligonucleotide of each pair hybridizes preferentially in each
case if in the genomic DNA sample the position was **methyalted**,
while the other oligonucleotide of the pair hybridizes preferentially, if
the position was **unmethyalted**, each oligonucleotide of a pair is. . .
used one determines the degree of polarization. An INDEPENDENT CLAIM is
also included for a diagnostic kit for detecting the **methylation**
of specific **cytosine** bases in genomic DNA samples comprising
one or more pairs of fluorescent labeled oligonucleotides designed to
hybridize to a target. . . oligonucleotide hybridization is detected
by an increase in fluorescence polarization. The fluorophore is a
5'-carboxyfluorescein, 6-carboxy-X-rhodamine, N,N,N',N'-tetramethyl-6-
carboxy-X-rhodamine, BODIPY, Texas Red, **Cy3**, **Cy5**,
FAM, fluorescein isothiocyanate (FITC), DAPI, HEX or TET. The DNA sample

is cleaved prior to bisulfite treatment with restriction endonucleases..
. . . steel, iron, copper, nickel, silver or gold, prior to hybridization
with the Type B oligonucleotide. The information generated about the
methylation status at the target site is provided to a computing
device comprising one or more databases or learning algorithms.

USE - (M) is useful in analysis of **methylation** of specific
cytosine bases in genomic DNA samples isolated from mammalian
sources e.g. cell lines, blood, sputum, feces, urine, cerebrospinal
fluid, tissue embedded. . . density DNA chip analysis, provides a
cost-effective method of analysis and the results are obtainable minutes
after carrying out the **methylation** specific reaction. (30
pages)

L18 ANSWER 3 OF 19 CAPLUS COPYRIGHT 2003 ACS on STN

ACCESSION NUMBER: 2003:633164 CAPLUS

DOCUMENT NUMBER: 139:160777

TITLE: Identification of **methyalted**
cytosines in DNA using differential reactivity
with bisulfite and using probes for parallel analysis
of many potential sites

INVENTOR(S): Garner, Harold R.; Minna, John D.; Luebke, Kevin J.;
Balog, Robert P.

PATENT ASSIGNEE(S): USA

SOURCE: U.S. Pat. Appl. Publ., 210 pp.

CODEN: USXXCO

DOCUMENT TYPE: Patent

LANGUAGE: English

FAMILY ACC. NUM. COUNT: 1

PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
US 2003152950	A1	20030814	US 2002-184085	20020627
PRIORITY APPLN. INFO.:			US 2001-301370P	P 20010627

TI Identification of **methyalted cytosines** in DNA using
differential reactivity with bisulfite and using probes for parallel
analysis of many potential sites

AB The present invention provides a high-throughput method for the parallel
anal. of many potential sites of chem. modification (e.g.,
methylation) in DNA. It makes use of chem. treatment of the DNA
to alter its sequence in a way that depends upon the modification of
interest and subsequent anal. of the resulting sequence by hybridization
to an array of probes. Treatment with sodium bisulfite converts
methyalted and **unmethyalted** DNA to different sequences.
Unmethyalted cytosines in DNA react with sodium bisulfite to
yield deoxyuridine, which behaves as thymidine in hybridization and

enzymic template-directed polyn., whereas **methyated cytosines** are unreactive and behave as **cytosine** in hybridization and polyn. A device, comprising the array of probes, is provided by the invention, and principles and methods for its design and fabrication are also provided. A procedure for probe design is provided in which a parent probe created from the complementary sequence is filtered to remove probes that are deemed not to be suitable for re-sequencing anal., and each parent probe is used as a template to create new probes to query for possible changes at a particular position in the ref. sequence. Thus, **methylation** is analyzed in a region of the promoter for the tumor suppressor gene p16. A 1280-member 20-mer probe set for a 145-bp region of the promoter of p16 is provided.

ST DNA **methylation** analysis bisulfite reactivity probe set; gene p16 **methylation** analysis bisulfite probe set